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REMARKS

Claim Amendments:

The claims have been amended to better describe the present invention. Support for the amendments is found in the specification as follows.

For Claims 1, 4, 58, and 62, support for the amendment or added claim is found on page 9, lines 20-22, and in the original claims.

For Claims 2, 5, 21, 36, 51, 55, 57, and 61, support for the amendment or added claim is found on page 8, lines 1-14; page 9, lines 12-17; and in the original claims.

Claim 3 has been amended to correct a typographical error.

For Claims 49, 50, 53, 54, 59, 60, 63 and 64, support for the added claims is found on page 9, lines 18-28.

All other amendments are merely clerical in nature and support for all other added claims is found in the original claims.

Objection to the Specification and Rejection of Claims 1, 4, 7, 39, 44, 47 and 48 Under 35 U.S.C. § 112, First Paragraph:

The Examiner has maintained the objection to the specification and the rejection of Claims 1, 4, 7, 39, 44, 47 and 48 under 35 U.S.C. § 112, first paragraph, for the reasons of record, on the basis of written description. With regard to Applicants' arguments in the response filed July 27, 2001, the Examiner contends that the evidence provided in the Weeks Declaration only provides sufficient written description of isolation of a dicamba-degrading oxygenase from *Pseudomonas* spp., and that the Weeks Declaration does not provide evidence that Applicant was in possession of dicamba tolerant transgenic plants at the time of the invention, or methods of using the plants.

Applicants traverse the rejection under 35 U.S.C. § 112, first paragraph. Initially, Applicants note that the claims have been amended to more particularly describe the present invention. Specifically, Claim 1 now recites a DNA sequence encoding a dicamba-degrading oxygenase from a dicamba-degrading bacterium, and Claim 2 is a sequence-based claim, wherein oxygenases having at least about 65% homology with SEQ ID NO:4 and having dicamba-degrading oxygenase activity are claimed. Other independent claims have been similarly amended or added. Applicants note that

claims directed to a dicamba-degrading oxygenase from *Pseudomonas* have been added, which Applicants believe the Examiner has indicated would be allowable.

With regard to the scope of the claims directed to DNA molecules and constructs, Applicants submit that the disclosure of the exemplified *Pseudomonas* species, further supported by the reference in the specification to publications describing other organisms that degrade dicamba, as well as the disclosure of the identification of additional dicamba-degrading oxygenases (see July 27, 2001 Declaration under 37 CFR 1.132 of Dr. Weeks), is a disclosure of a representative number of species sufficient to demonstrate that Applicants were in possession of the claimed genus of bacterial dicamba-degrading oxygenases at the time of the present invention. As set forth previously, the specification provides a substantial disclosure describing how to identify and isolate additional nucleic acid sequences encoding the same or different oxygenases (see, e.g., page 9, lines 18-28, page 10, line 10 through page 11, line 4, and Examples 1 and 2). As discussed in the specification, other dicamba-degrading bacteria were known at the time of the present invention, and the information in the present specification regarding the exemplified dicamba-degrading oxygenase can readily be used to identify and isolate dicamba-degrading oxygenases from other bacteria that degrade dicamba.

Indeed, the July 27 Declaration demonstrates that, using the procedures taught in the specification, several additional genes encoding dicamba-degrading oxygenases can be readily identified in other bacteria, such as in bacteria of the genus *Sphingomonas*. The Examiner states that at least two *Pseudomonas* species have been reclassified as *Sphingomonas* species, apparently in support of a position that Applicants are only entitled to claim dicamba-degrading oxygenases from *Pseudomonas* species. However, Applicants submit that the proper interpretation of this data is that Applicants have demonstrated the presence of structurally related dicamba-degrading oxygenases in at least two bacterial genera. This supports Applicants' position that the genus of dicamba-degrading oxygenases would not be expected to vary widely. As discussed in the specification (page 3, lines 12-21), other dicamba-degrading bacteria were known in the art at the time of the invention. Applicants respectfully refer the Examiner to one of the references cited in the specification on page 3, which is of record in Applicants' 1449 form (Krueger et al., 1989, *J. Agric. Food Chem.*, 37:534-538). As discussed in the abstract and at column 1, the first paragraph of the "Results and Discussion" section, eight different species of soil bacteria from *five different genera* were identified

that use dicamba as a sole carbon source (i.e., that degrade dicamba). Applicants submit that one of skill in the art would appreciate that bacteria that have the ability to degrade dicamba would have some common characteristics, such as might be found between the genera of *Pseudomonas* and *Sphingomonas*. Moreover, Applicants submit that it would be expected that such bacteria would contain a dicamba-degrading oxygenase that has structural and functional homology to the dicamba-degrading oxygenase described in the present invention. The Examiner has not given any reason why one of skill in the art would expect to find that dicamba-degrading oxygenases from other bacterial genera vary substantially in characteristics from the disclosed oxygenase. Indeed, as set forth in the July 27 Declaration, the newly-identified genes are greater than 90% identical to SEQ ID NO:3.

With regard to the sequence based claims, amended Claim 2 and similar claims now recite a specific level of identity to the disclosed sequence which a given sequence must have to be within the scope of the claims. The specification also describes how one can measure the sequence identity (page 9, lines 12-17). The procedures that can be used to make and identify DNAs that fall within the scope of this claim are conventional in the art and an assay is described to identify proteins having the claimed activity (see Example 1). There is not substantial variation in the genus because all members must have at least 65% identity to the reference sequence and must have dicamba-degrading oxygenase activity. The disclosed species are representative of the genus because all members have at least 65% structural identity with the recited sequence and because of the provision in the specification of assays to identify variants having the recited biological activity.

With regard to claims directed to transgenic host cells, transgenic plants, and methods of selecting and using such plants, Applicants submit herewith a new Declaration of Dr. Weeks under 37 CFR 1.132, which provides evidence that transgenic plants expressing a transgene encoding the dicamba-degrading oxygenase of the present invention are in fact tolerant to dicamba as compared to the non-transgenic controls. The experiments in the Declaration were performed in accordance with the teachings in the specification. This Declaration was not earlier presented because it was thought that the prior Declaration under this section would be sufficient to address the Examiner's concerns regarding written description and enablement. The data provided in the second Declaration

of Dr. Weeks confirm that, at the time of the present invention, Applicants were in possession of dicamba tolerant, transgenic plants and methods of making and using such plants.

In view of the foregoing discussion, Applicants respectfully request that the Examiner withdraw the rejection of Claims 1, 4, 7, 39, 44, 47 and 48 under 35 U.S.C. § 112, first paragraph.

Objection to the Specification and Rejection of Claims 39, 44, 47 and 48 Under 35 U.S.C. § 112, First Paragraph:

The Examiner has maintained the objection to the specification and rejection of Claims 39, 44, 47 and 48 under 35 U.S.C. § 112, first paragraph for the reasons of record on the basis of enablement. In response to Applicants' arguments filed on July 27, 2001, the Examiner contends that the state of the art at the time of the invention was such that producing transgenic plants having a specific phenotype such as tolerance to dicamba was unpredictable and required empiric evidence that the dicamba-degrading oxygenase confers dicamba tolerance to the plant. The Examiner states that the Weeks Declaration shows transgenic plants having dicamba-degrading oxygenase activity, but asserts that resistance to dicamba has not been shown.

Applicants traverse the rejection under 35 U.S.C. § 112, first paragraph. As discussed above, the new Declaration of Dr. Weeks under 37 CFR 1.132 demonstrates that transgenic plants expressing a transgene encoding the dicamba-degrading oxygenase of the present invention are tolerant to dicamba as compared to the non-transgenic controls.

In view of the foregoing discussion, Applicants respectfully request that the Examiner withdraw the rejection of Claims 39, 44, 47 and 48 under 35 U.S.C. § 112, first paragraph.

Objection to the Specification and Rejection of Claims 1, 4, 7, 21, 24 and 36 Under 35 U.S.C. § 112, First Paragraph:

The Examiner has maintained the rejection of Claims 1, 4, 7, 21, 24 and 36 under 35 U.S.C. § 112, first paragraph, for the reasons of record. In response to Applicants' arguments in the July 27 response, the Examiner contends that the teachings of the instant application are limited to a dicamba-degrading oxygenase from *Pseudomonas maltophilia*. The Examiner acknowledges that the Weeks Declaration teaches additional species that use dicamba as a carbon source.

*Sphingomonas* sp., the Examiner asserts that at least two *Pseudomonas* spp. have been reclassified as a *Sphingomonas* species. Therefore, the Examiner admits that the specification allows one to identify other *Pseudomonas* dicamba-degrading oxygenases without undue experimentation, but submits that it would require undue experimentation to isolate the oxygenases from the myriad of known species. The Examiner asserts that Applicant has not provided sufficient guidance as to what structural features one of skill in the art would have to look for to identify the genus of dicamba-degrading oxygenases.

Applicants traverse the rejection under 35 U.S.C. § 112, first paragraph. As discussed previously, the specification provides sufficient guidance to one of skill in the art to enable one of skill in the art to identify and isolate dicamba-degrading oxygenases from other bacteria and to identify, isolate or produce dicamba-degrading oxygenases and homologues thereof having the recited homology to the disclosed dicamba-degrading oxygenase sequence. Indeed, using the methods described in the specification, Applicants have demonstrated the identification of dicamba-degrading oxygenases from other bacterial species, including bacteria of a genus other than *Pseudomonas*, and including dicamba-degrading oxygenases having at least 65% sequence identity to SEQ ID NO:3.

The Examiner asserts that at least two *Pseudomonas* species have been reclassified as *Sphingomonas* species, apparently in support of a position that Applicants are only entitled to claim dicamba-degrading oxygenases from *Pseudomonas* species. However, as discussed above, Applicants submit that the proper interpretation of this data is that Applicants have demonstrated that one of skill in the art, using the guidance in the specification, can readily identify dicamba-degrading oxygenases in other bacterial species and indeed, in more than one genus of bacteria. To the extent that the two genera of bacteria, *Pseudomonas* and *Sphingomonas*, are related by common characteristics, Applicants submit that one of skill in the art would naturally look to bacteria having similar characteristics to identify other dicamba-degrading bacteria. Indeed, the Krueger et al. reference discussed above shows that soil bacteria from several different genera were known in the art at the time of the invention to use dicamba as a sole carbon source, all of which would serve as a source from which to identify other dicamba-degrading oxygenases. To limit Applicants to dicamba-degrading oxygenases from *Pseudomonas* is not a fair or reasonable acknowledgment of

the claim scope to which Applicants are entitled, particularly given the clear demonstration of the identification of dicamba-degrading oxygenases in a bacterial genus other than *Pseudomonas*.

One of skill in the art may expect that it may take some time and experimentation to isolate dicamba-degrading oxygenases from other bacteria, but the law is clear that an extended period of experimentation and optimization is not necessarily undue, provided that the specification gives a reasonable amount of guidance with respect to the direction the experimentation should proceed. (See MPEP 2164.06) For example, for those of skill in the art working with microorganisms, screening many of microbial colonies (i.e., even thousands) in *a single experiment* is *routine*. The specification has shown those of skill in the art the structural and functional characteristics of a dicamba-degrading oxygenase and has taught how to use this information to identify additional dicamba-degrading oxygenases falling within the scope of the claims, as amended. Applicants submit that, given the guidance in the specification, one of skill in the art is not left with an infinite number of possible choices, but a well-directed strategy for isolating other dicamba-degrading oxygenases meeting the claim limitations.

In view of the foregoing discussion, Applicants respectfully request that the Examiner withdraw the rejection of Claims 1, 4, 7, 21, 24 and 36 under 35 U.S.C. § 112, first paragraph.

Applicants have attempted to respond to all of the Examiner's concerns and submit that the claims are in a condition for allowance. If the Examiner has any questions or concerns regarding Applicants' position, contact of the below-named agent is encouraged.

Respectfully submitted,

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09/055,145



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In the Claims:

Claims 1-5, 7, 21, 24, 36, 38, 39, 44, 47, and 48 have been amended as shown below.

Claims 6, 22, 23, and 37 remain unchanged.

Claims 49-64 have been added.

1. (Twice Amended) An isolated DNA molecule comprising a DNA sequence encoding a dicamba-degrading oxygenase from a dicamba-degrading bacterium.

2. (Twice Amended) An isolated [The] DNA molecule [of Claim 1] comprising a DNA sequence encoding a dicamba-degrading oxygenase, wherein said dicamba-degrading oxygenase is selected from the group consisting of:

a. a dicamba-degrading oxygenase having the amino acid sequence of SEQ ID NO:4; and

b. a dicamba-degrading oxygenase having an amino acid sequence which is at least about 65% identical to the amino acid sequence of SEQ ID NO:4 and which has dicamba-degrading oxygenase activity.

3. (Once Amended) The DNA molecule of Claim 2 comprising the nucleotide sequence of SEQ ID NO:[2]3.

4. (Twice Amended) A DNA construct comprising a DNA sequence encoding a dicamba-degrading oxygenase from a bacterium that degrades dicamba operatively linked to expression control sequences.

5. (Twice Amended) A [The] DNA construct [of Claim 4] comprising a DNA sequence encoding a dicamba-degrading oxygenase operatively linked to expression control sequences, wherein said dicamba-degrading oxygenase is selected from the group consisting of:

a. a dicamba-degrading oxygenase having the amino acid sequence of SEQ ID NO:4; and

b. a dicamba-degrading oxygenase having an amino acid sequence which is at least about 65% identical to the amino acid sequence of SEQ ID NO:4 and which has dicamba-degrading oxygenase activity.

7. (Once Amended) The DNA construct of Claim 4 or 5 which is a vector.

21. (Twice Amended) A transgenic host cell comprising DNA encoding a dicamba-degrading oxygenase, said DNA being operatively linked to expression control sequences;

wherein said dicamba-degrading oxygenase is selected from the group consisting of:

a. a dicamba-degrading oxygenase having the amino acid sequence of SEQ ID NO:4; and

b. a dicamba-degrading oxygenase having an amino acid sequence which is at least about 65% identical to the amino acid sequence of SEQ ID NO:4 and which has dicamba-degrading oxygenase activity.

24. (Once Amended) The transgenic host cell of Claim 21 or 58 which is a plant cell.

36. (Twice Amended) A transgenic plant or part of a plant comprising one or more cells comprising DNA encoding a dicamba-degrading oxygenase, said DNA being operatively linked to expression control sequences;

wherein said dicamba-degrading oxygenase is selected from the group consisting of:

a. a dicamba-degrading oxygenase having the amino acid sequence of SEQ ID NO:4; and

b. a dicamba-degrading oxygenase having an amino acid sequence which is at least about 65% identical to the amino acid sequence of SEQ ID NO:4 and which has dicamba-degrading oxygenase activity.

38. (Once Amended) The transgenic plant or plant part of Claim 37 wherein the DNA comprises the [nucleotide] nucleotide sequence of SEQ ID NO:3.

39. (Once Amended) The transgenic plant or plant part of Claim 36 or 62 wherein the plant is a broadleaf plant which is tolerant to dicamba as a result of the expression of the

dicamba-degrading oxygenase and the plant part is a part of a broadleaf plant which is tolerant to dicamba as a result of the expression of the dicamba-degrading oxygenase.

44. (Twice Amended) A method of controlling weeds in a field containing a transgenic plant according to any one of Claims 36-39 or 61-64 comprising applying an amount of dicamba to the field effective to control the weeds in the field.

47. (Twice Amended) A method of selecting transformed plant cells comprising:  
providing a population of plant cells;  
transforming at least some of the plant cells in the population of plant cells with a DNA construct according to any one of Claims 4-7 or 53-56; and  
selecting the transformed plant cells by culturing the resulting population of plant cells in a culture medium containing dicamba at a concentration selected so that transformed plant cells proliferate and untransformed plant cells do not proliferate.

48. (Twice Amended) A method of selecting transformed plants comprising:  
providing a population of plants which may comprise one or more plants comprising a DNA construct according to any one of Claims 4-7 or 53-56; and  
selecting transformed plants by applying an amount of dicamba to the population of plants selected so that transformed plants grow, and growth of untransformed plants is inhibited.



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In re the Application of:

WEEKS et al.

Serial No.: 09/055,145

Filed: April 3, 1998

Atty. File No.: 3553-18

For: "METHODS AND MATERIALS FOR  
MAKING AND USING  
TRANSGENIC DICAMBA-  
DEGRADING ORGANISMS"

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) Group Art Unit: 1638

) Examiner: D. Kruse

DECLARATION OF  
DR. DONALD P. WEEKS  
(Under 37 CFR 1.132)

TECH CENTER 1600/2900

Assistant Commissioner for Patents  
Washington, D.C. 20231

Dear Sir:

I, Dr. Donald P. Weeks, declare that:

1. I am the same Donald P. Weeks who is named as an inventor on the above-referenced patent application.
2. Experiments have been performed in my laboratory to demonstrate that the expression of a dicamba-degrading oxygenase by a transgenic plant confers dicamba tolerance to the transgenic plant as compared to a non-transgenic plant. The experiments were performed as follows.

Tobacco plants were transformed with a construct encoding the dicamba-degrading oxygenase from *Pseudomonas maltophilia* strain DI-6 (SEQ ID NO:4) and the same gene construct to which a DNA sequence encoding a transit peptide had been added. In treatments of transgenic plants containing either construct with various doses of dicamba, plants containing either construct showed resistance to dicamba at concentrations of at least 2.5. lbs/acre.

Briefly, to create the oxygenase gene bearing the transit peptide, the coding region of the oxygenase gene was modified to contain at its 5' end a sequence encoding a transit peptide that would allow targeting of the protein product of the modified gene to the chloroplast or other organelles of transgenic plants. As one specific example, polymerase chain reaction (PCR) was used to amplify a DNA region encoding the pea ribulose 1,5-bisphosphate carboxylase small subunit transit peptide (signal) sequence for insertion into a pRTL2 vector containing the oxygenase gene. The PCR primers were designed to insert an Nco I-restriction enzyme cut site at either end of the amplified transit peptide coding sequence and

to fuse the transit peptide coding region in frame with the coding region of the oxygenase gene. Thus, the amplified fragment was digested with Nco I and ligated into the Nco I cut site at the ATG initiation codon of the genetically engineered oxygenase gene contained in vector pRTL2. The entire cassette (TEV leader, the transit peptide and the oxygenase gene) was then cut out of the pRTL2 vector as an XhoI/XbaI fragment and introduced into the binary vector pKLP36 containing the PCISV FLt36 promoter and the rbcS 3' terminator. The construct was moved into the *A. tumefaciens* strain C58C1 by a modified triparental mating procedure routinely used by the UNL Plant Transformation Core Research Facility. This involved incubating *Escherichia coli* cells carrying each construct with a mixture of *A. tumefaciens* cells and *E. coli* cells carrying the helper plasmid pRK2013 (for plasmid mobilization).

Tobacco plants were transformed by incubating leaf explants with a suspension of *A. tumefaciens* cells containing the oxygenase construct, followed by regeneration of shoots on solid medium containing kanamycin (Horsch et al., *Science*, 227:1229-1231 (1985)). Ten shoots were selected from the transformation, placed on rooting medium for a few weeks, and then moved to pots in the greenhouse.

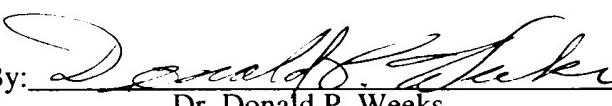
To test the resistance of the transgenic tobacco plants to dicamba, seeds from the T<sub>0</sub> generation were germinated and grown to the 10-12-leaf stage. With the assistance of the Department of Agronomy, these plants were subjected to 'constant rate spraying' using a custom made sprayer (Burnside, Weed Science Vol.17, No.1, 102-104, 1969), manufactured by ISCO. The sprayer has a TEEJET nozzle and screen, uses a delivery speed of 1.87 mph and a pressure of 42 PSI. The nozzle height is set at 8" above canopy. Dicamba (commercial product-Clarity), at various concentrations, was sprayed on the plants mixed with a non-ionic surfactant at a rate of 20 gallons per acre under compressed air.

To establish an initial kill curve, wild type tobacco plants were treated with increasing concentrations of dicamba ranging from 0 to 0.5 lbs/acre. Having established 100% sensitivity of the wild type plants to 0.5 lbs/acre of dicamba, transgenic plants containing the oxygenase gene were treated with increasing concentrations of dicamba ranging from 0.5 lbs/acre to 2.5 lbs/acre. The transgenic tobacco plants grew and were healthy when sprayed with a concentration of dicamba of up to 2.5 lbs/acre. That is, transgenic plants containing the oxygenase gene constructs appeared similar to the untreated control plants, whereas the non-transgenic plants treated with the same amounts of dicamba showed reduced growth, withering and severe necrosis. These results clearly demonstrate that the transgenic

plants transformed with the dicamba-degrading oxygenase gene constructs of the present invention have acquired resistance to dicamba.

3. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: February 14, 2002

By:   
Dr. Donald P. Weeks